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Population genetics and phylogeography of endangered *Oxytropis campestris* var. *chartacea* and relatives: arctic-alpine disjuncts in eastern North America

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Abstract

Fassett's locoweed (*Oxytropis campestris* var. *chartacea*, Fabaceae) is an endangered perennial endemic to Wisconsin. Patterns of genetic variation within and among six remaining populations and their relationship to other members of the *O. campestris* complex were analysed using AFLPs from 140 accessions across northern North America. Within-population measures of genetic diversity were high (mean expected heterozygosity $H_E = 0.16$; mean nucleotide diversity $\pi = 0.015$) compared with other herbaceous plants. Estimates of among-population differentiation were low ($F_{ST} = 0.12$; $\Phi_{ST} = 0.29$), consistent with outcrossing. Genetic and geographical distances between populations were significantly correlated within Fassett's locoweed ($r^2 = 0.73$, $P < 0.002$ for Mantel test) and *O. campestris* as a whole ($r^2 = 0.63$, $P < 0.0001$). Individual and population-based phylogenetic analyses showed that Fassett's locoweed is monophyletic and sister to *O. campestris* var. *johannensis*. Morphometric analyses revealed significant differences between Fassett's locoweed and populations of var. *johannensis*. The first chromosome count for Fassett's locoweed indicates that it is tetraploid ($2n = 32$), unlike hexaploid var. *johannensis*. High within-population diversity and relatively low among-population differentiation are consistent with populations of Fassett's locoweed being relicts of a more continuous Pleistocene distribution. Our data support the continued recognition of Fassett's locoweed and protection under federal and state regulations. High levels of genetic diversity within populations suggest that maintaining the ecological conditions that favour the life cycle of this plant may be a more pressing concern than the erosion of genetic variation.

Keywords: AFLPs, arctic-alpine plant, conservation, genetic variation, population genetics, *Oxytropis campestris*

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Introduction

Fassett's locoweed (*Oxytropis campestris* var. *chartacea* — Fabaceae), an endangered perennial herb with a basal leaf rosette, has been reported from only 10 sites, all sandy shorelines of small, fluctuating, usually calcareous lakes in Wisconsin. In 1998, Fassett's locoweed was found on only six of these sites, each disjunct from the others (Fig. 1) and from the remaining varieties of the circumboreal, arctic-alpine species to which it belongs (Fig. 2). Sites that it no longer occupies lack the sparsely vegetated, sandy shorelines needed for survival, reflecting the recent input of silt and nutrients from agricultural and residential development.

The federal recovery plan for Fassett's locoweed (USF & WS 1991) calls for data on the distribution of genetic variation within and among the remaining populations. Small, isolated populations are prone to genetic drift and inbreeding, which can erode genetic diversity, reduce fitness, and threaten the long-term survival of populations even in the absence of habitat destruction (Ellstrand & Elam 1993; Frankel *et al*. 1995). Populations of Fassett's locoweed also undergo severe fluctuations in size. Records kept by the Wisconsin Department of Natural Resources (DNR) since 1988 and many additional years of anecdotal evidence indicate that the size of individual populations can vary from tens of thousands of individuals to fewer than

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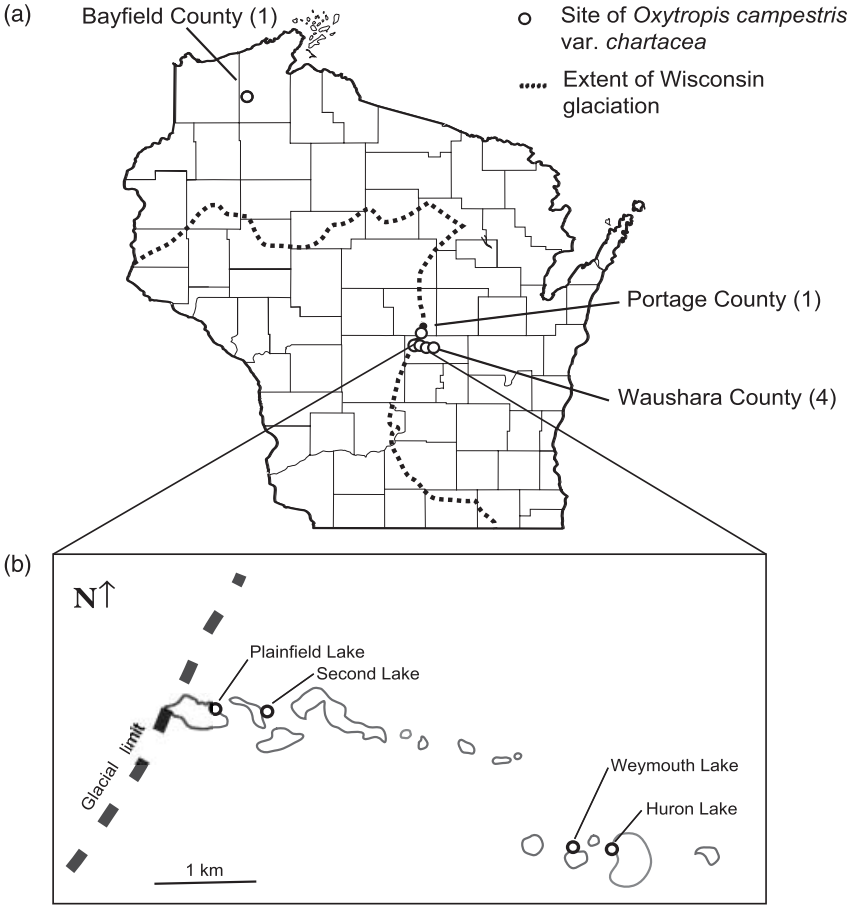

Fig. 1 Distribution of the six extant populations *Oxytropis campestris* var. *chartacea* in Wisconsin, with the number of sampled populations indicated in parentheses. Inset: location of the four Waushara County populations, on the shorelines of a line of kettle lakes associated with a tunnel channel in the ice at the glacial margin.

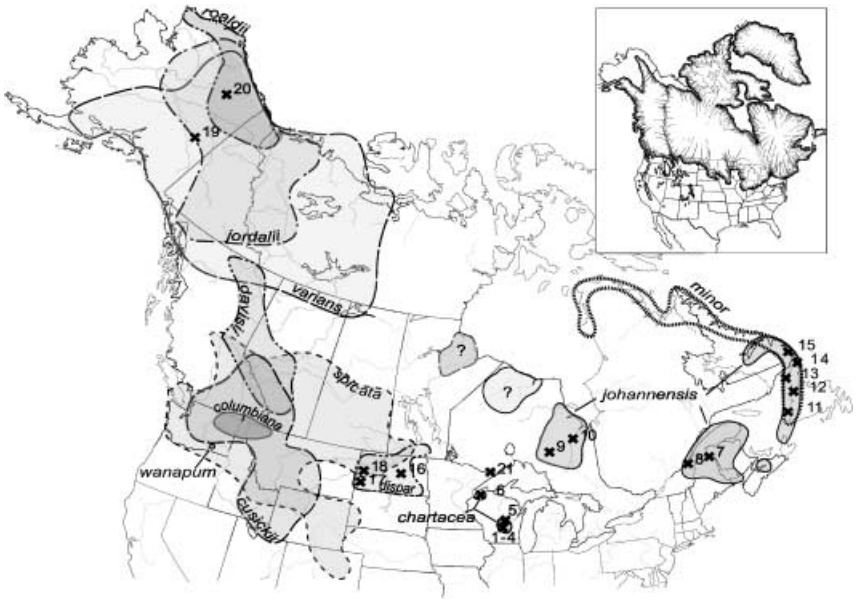

Fig. 2 Approximate distribution of the 12 varieties of *Oxytropis campestris* currently recognized. Sampled sites are indicated with an X and a reference number corresponding to Table 2 (sources: Barneby 1952; Scoggan 1957; Moss 1983; Ontario Ministry of Natural Resources; NDA; Welsh 2001). Inset: Maximum extent of glaciation in North America, including the Laurentide and Cordilleran ice sheets.

20 from year to year, making loss of genetic diversity as a result of bottlenecks an additional cause for concern. Knowledge of the extent and distribution of genetic variation is crucial for managing the existing populations and guiding the choice of propagation material with which to initiate new populations should that become necessary.

The taxonomic status of Fassett's locoweed is another key issue. Like most western or arctic/alpine elements

Table 1 Characteristics of the 12 North American varieties of *Oxytropis campestris*

Taxon	2*n*	Range	Habitat	Elevation (m)	Petal color	Pod length (mm)	Flower length (mm)	No. flowers per raceme	No. leaflets per leaf
varians (all)	16, 32, 48, 96	Northwest N.A.	Gravelly sites	15–2000	yellow-white	12–19	12–17	6–25	13–45
varians (2*n* = 48)	48	Northwest N.A.	Rocky hillsides, shores, roadsides	—	yellow-white	—	10–12.5	10–25	13–45
varians (2*n* = 96)	96	Northwest N.A.	Roadsides, gravel bars, open forest	—	yellow-white	—	11–15	8–15	13–23
jordalii	32	Northwest N.A.	Alpine tundra	10–1280	yellow-white	9–12	10–14	6–14	9–25
roaldii	64 ?	Northwest N.A.	Alpine/arctic tundra	5–450	lavender/ pink-purple	9–15	13–16	?	11–21
davisii	32	Canadian Rockies	Gravelly sites	915–1525	pink-purple	10–14	14–19	10–16	31–51
spicata	32, 48, 64	western N.A.	Prairies	1190–2260	yellow-white	12–23	12–19.5	6–30	17–33
cusickii	48	western N.A.	Rock outcrops, alpine meadows	2135–3355	yellow-white	10–19	14–18	6–15	7–17
columbiana	48	WA and MT	River shores	305–1060	white	16–23	15–20	6–28	11–17
wanapum	?	WA	Sandy ridge	600	pale lavender	13–23	14–20	6–12	19–26
dispar	32	ND	Prairies, buttes	5–1000	polychrome	13–18	17–19	8–15	19–25
minor	48	eastern N.A.	Cliffs/coastal tundra	0–520	pink-purple	10–22	11–18	3–9	11–27
johannensis	48	eastern N.A.	Rock outcrops/gravel bars	15–400	pink-purple	14–27	12–18.5	7–14	17–29
chartacea	32	WI	Sandy lakeshores	300–400	pink-purple	8–15	13–15	10–20	21–27

References: Barneby (1952); Welsh (2001); Dawe and Murray (1981); Elisens and Packer (1980).

in eastern North America, Fassett's locoweed was first described as a distinct species, *Oxytropis chartacea* (Fassett 1936), but was later reduced — in this case to a variety of *O. campestris* (Barneby 1952). *O. campestris*, with 12 recognized varieties (Welsh 2001; Table 1), represents a polyploid complex (2*n* = 16, 32, 48, 64, 96; Welsh 2001) with a circumboreal distribution centred on Beringia. While keeping Fassett's locoweed as a variety, Barneby suggested that future study may prove it to be indistinct from *O. campestris* var. *johannensis* from eastern Canada (Table 1; Fig. 2), which Barneby found to differ only in pod length and vesture. The recent discovery of specimens in western Ontario classified as *O. campestris* var. *johannensis* that resemble Wisconsin populations in pod length appears to weaken the argument for recognizing var. *chartacea* as a distinct entity (Welsh 1991, 1995, 2001; Isely 1998). A resolution of the genetic distinctiveness of var. *chartacea* would have a strong bearing on the legal protection afforded it at the federal and state levels.

A better understanding of the relationship of Fassett's locoweed to other members of the species and the genetic structure of its populations may also illuminate the origins of this unusual arctic-alpine element in the Wisconsin flora, one of very few taxa endemic to the state. All populations exist on recently glaciated territory, although most are immediately adjacent to the unglaciated Driftless Area. Do patterns of genetic variation suggest that the current populations are the product of recent long-distance dispersal, or are they relicts of the tundra flora that existed in Wisconsin during the late Pleistocene as the glaciers receded? In the former case we would expect low, within-population diversity, evidence of genetic bottlenecks, and little differentiation from possible source populations; in the latter, we would expect higher levels of genetic diversity and among-population variation conforming to an isolation-by-distance model.

We chose AFLPs (amplified fragment length polymorphisms) to address these issues. AFLPs are an extremely powerful probe of genetic variation, producing more information per primer than any other marker now in use (Karp *et al*. 1996; McGregor *et al*. 2000). AFLP bands have very high reproducibility (Vos *et al*. 1995; Jones *et al*. 1997), and have proven useful for analysing genetic variation within and among populations (Travis *et al*. 1996; Russell *et al*. 1999) and inferring phylogenetic relationships within and among closely-related species (Heun *et al*. 1997; Vijverberg *et al*. 2000).

Based on a survey of AFLP variation within Fassett's locoweed and other varieties of the *O. campestris* complex, we assess (i) how genetic diversity is distributed within and among populations of Fassett's locoweed, and whether

bottlenecks appear to have severely eroded genetic variation; (ii) the degree to which Fassett's locoweed is genetically distinct from other taxa; and (iii) what these results imply about possible scenarios of diversification within the complex and their relationship to geological and glacial history. We also use chromosome counts and morphometry to address these questions.

Materials and methods

AFLPs

Leaf material was collected from all six populations of Fassett's locoweed with plants above-ground in 1998–1999. Twenty-five plants per population were sampled from the three large populations. All plants were sampled in two populations with only four plants and a third with 14 (Table 2). A total of 20 individuals of *O. campestris* var. *johannensis* was sampled from three regions in Maine and Quebec, Newfoundland and Labrador, and eastern Ontario near James Bay (Fig. 2). Other varieties of *O. campestris* sampled included var. *dispar* and var. *spicata* from North Dakota, the nearest populations west of Wisconsin, and more distant vars. *jordalii* and *varians* from Alaska (Fig. 2). Var. *varians*, with the only diploid chromosome counts in the species, its highly variable morphology and proximity to the Beringia region, has been suggested to represent ancestral, 'proto-*campestris*' stock (Dawe & Murray 1981). For outgroups, we included samples of *Oxytropis borealis* var. *viscida* from northern Minnesota and two distantly related species, *O. lambertii* and *O. deflexa*, from Minnesota and Labrador, respectively (Table 2). Voucher specimens are deposited at WIS.

In each population, leaves were collected from individual plants spaced as widely apart as possible. Plants at Plainfield Lake in Wisconsin were sampled at *c*. 30 m intervals. Harvested tissue was placed immediately in silica gel or, for fresh or frozen samples, on ice until they could be returned to the lab for DNA extraction or storage at −80 °C. Leaf samples from plants from the St. John River in Maine and the St. Lawrence River in Quebec were provided by Sue Gawler (Maine Natural Areas) and Marcel Blondeau (Université Laval). All samples from Alaska, preserved in silica gel, were provided by Jan Jorgensen (University of Alaska-Fairbanks).

DNA was extracted from leaves using one of three methods: (i) DNeasy Plant Mini Kits (Qiagen) using 100 mg frozen or 25 mg silica gel-dried tissue; (ii) DNeasy 96 Plant Kits for high-throughput DNA extraction using 50 mg fresh material (Myburg & Remington 2000); or (iii) a modified 6x CTAB extraction protocol using 100 mg frozen leaf tissue (Doyle & Doyle 1988). The method used for individual samples is indicated in Table 2. AFLP profiles for individual samples clustered in the same population as others extracted using different techniques, suggesting that extraction technique did not have a material effect.

Standard AFLP protocols [Myburg & Remington (2000) following Vos *et al*. (1995)] were carried out in the laboratory of the Forest Biotechnology Group of North Carolina State University. For each sample, 100–500 ng total DNA was digested with *Eco*RI and *Mse*I followed by ligation with adapters (*Eco*RI adapter = 5′-GACTGCGTACCAATTC-3′; *Mse*I adapter = 5′-GATGAGTCCTGAGTA-A-3′). Selective pre-amplification was carried out using *Eco*RI and *Mse*I primers consisting of adapter sequences plus one additional selective nucleotide (*Eco*RI + A and *Mse*I + C). PCR (polymerase chain reaction) reaction volumes totalled 30 μL including 3 μL restriction–ligation mixture diluted 1:10 in deionized water as template, 2 units *Taq* polymerase (Qiagen), 50 ng *Eco*RI and *Mse*I primers, 0.5 mM $MgCl_2$, 0.2 mM dNTPs, and 1 × PCR buffer. PCR amplifications were began at 72 °C for 30 s followed by 28 cycles denaturation at 94 °C for 15 s, 30 s annealing at 60 °C, and 1 min (+1 s/cycle) extension at 72 °C. Final amplifications used 3 μL of pre-amplification product diluted 40:1 in deionized water as template, 0.6 units *Taq* polymerase, 3 ng infrared dye (IRD)-labelled *Eco*RI primer (Li-Cor), 15 ng *Mse*I primer, 0.2 mM dNTPs, and 1 × PCR buffer in 30 μL total volume. *Eco*RI primer sequences included three selective nucleotides beginning with A; *Mse*I primers included three or four selective nucleotides beginning with C. The PCR amplification consisted of 13 cycles of denaturation at 94 °C for 10 and 30 s at 65° (–0.7 °C per cycle), and 1 min at 72 °C; followed by 25 cycles of 10 s at 94 °C, 30 s at 56 °C, 1 min (+1 s per cycle) at 72 °C. Twenty-two primer pairs were screened for their ability to produce scorable bands. Of these, four primer pairs (*Mse*I/*Eco*RI) CCAA/ACG, CGC/ACG, CTG/ACG, CGC/ATG, were used in the final analysis.

AFLP reaction products were resolved on denaturing gels containing 8% Long Ranger polyacrylamide, 7.0 M urea, and 0.8 × TBE buffer. Loading buffer (5–7 μL) consisted of 95% deionized formamide, and 20 mM ethylene diamine tetra acetate (EDTA). Prior to gel loading, 1 mg/mL bromophenol blue was added to each final amplification product; the mixture was heated at 90 °C for 3 min, then quickly cooled on ice before loading 1 μL of each sample. Molecular-weight markers (Li-Cor) were loaded in two lanes as a standard. Electrophoresis was carried out on Li-Cor 4000 L automated sequencers using 0.8 × TBE running buffer, with run parameters of 1500 V, 35 mA, 42 W, signal channel 3, motor speed 3, 48 °C plate temperature and 16-bit pixel depth for collection of TIFF image files.

Same-size fragments were scored as present or absent by eye from the TIFF image files using AFLP Quantar (Keygene). A subset of samples (12–122) per primer pair (Table 3) was replicated from the final amplification stage onward, resulting in a total of 243 replications across all primer pairs. In addition to these amplification replicates,

Table 2 Site codes (see Fig. 2), scientific names, sample size, locality, coordinates, habitat and extraction protocol (1= DNeasy/frozen or silica-gel dried; 2 = DNeasy, fresh leaves; 3 = 6X CTAB, frozen leaves) for populations sampled for this study

Site code	Scientific name	*N*	State/ Prov.	Locality	Lat./Long.	Habitat	Voucher specimen	Extraction protocol
1	*Oxytropis campestris* var. *chartacea* (Fassett) Barneby	25	WI	Waushara Co., Plainfield Lake	44°12′26″ N 89°28′13″ W	Sandy lakeshore; fluctuating, calcareous	v0187967WIS	2
2	″″	14	WI	Waushara Co., Second Lake	44°12′25″ N 89°27′51″ W	Sandy lakeshore; fluctuating, calcareous lake	v0187969WIS	2
3	″″	25	WI	Waushara Co., Weymouth Lake	44°11′43″ N 89°25′51″ W	Sandy lakeshore; fluctuating, calcareous lake	v0187970WIS	2
4	″″	4	WI	Waushara Co., Huron Lake	44°11′43″ N 89°25′09″ W	Sandy lakeshore; fluctuating, calcareous lake	v0187972WIS	1, 2
5	″″	4	WI	Portage Co., Pickerel Lake	44°19′03″ N 89°19′15″ W	Sandy lakeshore; fluctuating, calcareous lake	v0187971WIS	1, 2
6	″″	25	WI	Bayfield Co., Mountain Lake	46°20′48″ N 91°22′21″ W	Sandy lakeshore; fluctuating	v0187973WIS	2, 3
7	*Oxytropis campestris* var. *johannensis* Fernald	3	ME	St. John River, Ft. Kent	47°14′20″ N 68°42′ W	Slate ledge	Sue Gawler 99-014	1
8	″″	1	QUE	St. Lawrence River, Lévis	46°50′ N 71°07′ W	Rocher du haut rivage	QFA S96016A	1
9	″″	4	ONT	Missanaibi River, Mattice	49°45′ N 83°30′ W	Gravelly river shore (dolomitic)	WIS 90617, 90627	1
10	″″	2	ONT	Moose River, Moose River Crossing	50°50′ N 81°10′36″ W	Sandy (gypsum) River shore	WIS 90625	1
11	″″	2	NF	Cape St. George	48°46′ N 59°26′ W	Limestone sea cliff	WIS 90622	1
12	″″	2	NF	Cow's Head	49°55′ N 57°50′ W	Limestone sea cliff	WIS 90623	1
13	″″	2	NF	Pointe Riche	50°42′ N 57°24′ W	Road gravel	WIS 90618	1
14	″″	2	NF	Burnt Cape	51°35′ N 55°44.5′ W	Limestone sea cliff	WIS 90619	1
15	″″	2	LAB	L'Anse Amour	51°27.5′ N 56°52.5′ W	Rocky seashore	WIS 90616	1
16	*Oxytropis campestris* var. *dispar* (A. Nelson) Barneby	4	ND	Wells CO., Frederick WMA	47°20′30″ N 100° W	Shortgrass prairie	WIS 90600–90603	1
17	″″	2	ND	Billings CO., Medora	46°55′ N 103°30′ W	Badlands	WIS 90604–90605	1
18	*Oxytropis campestris* var. *spicata* Hooker	4	ND	Billings CO., Fairfield	47°9′45″ N 103°13′30″ W	Limestone outcrop, Shortgrass prairie	WIS 90610–90613	1
19	*Oxytropis campestris* var. *varians* (Rydberg) Barneby	2	AK	Big Delta Quad, Birch Lake	64°20′ N 146°40′ W	Roadside gravel	(JJ99-12 see ALA V129392–3)	1
20	*Oxytropis campestris* var. *jordalii* (Porsild) S. Welsh	2	AK	Mt. Hultén	68°26′ N 149°21′ W	Sedge -*Dryas* tundra, coarse sandy soil	ALA V129342	1
18	*Oxytropis lambertii* var. *lambertii*	4	ND	Billings CO., Fairfield	47°9′45″ N 103°13′30″ W	Shortgrass prairie	WIS 90606–90609	1
21	*Oxytropis borealis* var. *viscida* (Nuttall) S. Welsh	2	MN	Cook CO., South Fowl Lake	48°02′55″ N 90°0′27″ W	Slate cliff overlooking river	v0187968WIS	1
15	*Oxytropis deflexa* var. *foliolosa* (Hooker) Barneby	2	LAB	L'Anse Amour	51°27′30″ N 56°52′30″ W	Roadside gravel	WIS 90621	1

Table 3 Primer combinations used for selective amplification, number of scorable loci, number of samples replicated from final amplification, scoring mismatch rate between samples replicated from final amplification and scoring mismatch rate for three samples replicated from leaf collection

Mse primer	EcoRI primer	No. of loci	No. of replicated samples	Mismatch rate between replicates	Mismatch rate among complete replicates
CGC	ACG	56	122	0.039	0.033
CTG	ACG	50	12	0.035	0.047
CCAA	ACG	64	15	0.022	0.035
CGC	ATG	40	94	0.030	0.032
	Total:	210	243	Ave: 0.032	0.037
				SD: 0.007	0.007

three sets of complete replicates were included by sampling different leaves from the same three individuals on different occasions and bringing each set of samples separately through the extraction, amplification, and scoring protocol.

Analysis

Genetic variation. To obtain allele frequencies, populations were assumed to be in Hardy–Weinberg equilibrium. Null-allele frequencies and their variances were estimated using a Bayesian approach (Zhivotovsky 1999) employing a Matlab program, DMAP (Dominant Marker Analysis Package), written by Greg Gelembiuk. The parameters of an informative beta prior were estimated from band frequencies across the set of sampled populations (Zhivotovsky 1999). Because the sample size of several populations was quite small (i.e. $n = 2$) such populations were grouped for some analyses to form larger sample sizes with which to obtain more stable estimates of gene frequencies. Pooling was based on geographical proximity and evidence of clustering on trees of individuals.

Heterozygosity and its variance was calculated using the asymptotically unbiased estimator of Lynch & Milligan (1994) from allele frequencies estimated as described above. Two additional measures of within-population genetic diversity were also calculated: nucleotide diversity (π) — the average number of nucleotide differences per site between two randomly chosen DNA sequences in a population — was estimated as a function of the proportion of mismatched bands between two individuals and the number of discriminating sites in the amplification system (Borowsky 2001). Each band is assumed to represent a certain number of screened nucleotides; for AFLPs, this is equal to the number of bases in each restriction site plus those used in selective amplification. Hardy–Weinberg equilibrium is also assumed as well as a breeding population size of over 1000 individuals. This method has been shown to give stable estimates of diversity in surveys of balitorid hill-stream loaches from as few as two individuals using 11 primers and 1000 loci (Borowsky 2001). Diversity was also estimated using a band-sharing coefficient which estimates within-population homogeneity using the similarity index of Lynch (1990). Similarity is calculated as

$$S_{xy} = 2N_{xy}/(N_x + N_y),$$

where N_{xy} is the number of pairs of bands matching across individuals x and y, and N_x and N_y are the total number of bands expressed by each individual (Lynch 1990). Leonard *et al*. (1999) provides an unbiased estimate of variance for mean S within a population. Diversity (dissimilarity) is expressed as $D = 1 - S$. Because size homoplasy resulting from comigrating nonhomologous bands can be more frequent among short AFLP fragments (Vekemans *et al*. 2002; Mechanda *et al*. 2004), diversity measures were also calculated using only bands greater than 250 bp in length. The number of singleton and private bands was also recorded. In order to compare the numbers of singleton and private bands between var. *chartacea* and var. *johannensis* despite the large differences in sample size, 1000 bootstrapped samples of $n = 20$ were taken from var. *chartacea*.

F-statistics (Wright 1951) were estimated following Lynch & Milligan (1994). We calculated both global and pairwise F_{ST} among all populations of *O. campestris*. Bootstraps were used to assess the significance of pairwise F_{ST} values. Given that estimation of gene frequencies from dominant markers requires the assumption of Hardy–Weinberg equilibrium, a phenetic method — analysis of molecular variance (AMOVA) — was also executed using Arlequin vs. 2.000 (Schneider *et al*. 2000). The number of migrants per generation (Nm) was calculated from F_{ST}'s according to the equation $Nm = (1 - F_{ST})/4F_{ST}$ (Wright 1969). Because estimates of gene flow based on an island model may be unrealistic, Nm was also estimated using a two-dimensional stepping stone model involving the regression of pairwise F_{ST} values against the logarithm of geographical distance between them (Rousset 1997). In addition, the statistic F (the probability that two genes share a common ancestor within a population) and its sampling variance was estimated for Wisconsin populations

via a Bayesian Markov chain Monte Carlo approach analogous to that of Holsinger *et al.* (2002), using the Matlab program DMAP. This statistic reflects population differentiation and provides a per-population measure of the relative balance of gene flow vs. drift, under the assumption of immigration-drift equilibrium (e.g. see Ciofi *et al*. 1999).

Spatial autocorrelation. To test for a correlation between genetic distance and geographical distance, permutation tests (Mantel 1967) were performed on the matrices of genetic and geographical distances between pairs of populations. Both Nei's (1972) distance and Cavalli-Sforza & Edwards (1967) chord distance were used for this analysis.

Phylogenetics. Clustering methods based on estimated genetic distances were used to infer phylogenetic relationships among individuals and populations. For individual-based analyses, the band presence/absence data matrix was directly converted into distance matrices using Jaccard's coefficient in PHYLIP (Felsenstein 1985) and the distance measure of Nei & Li (1979) in PAUP (Swofford 1993). Trees were constructed from both matrices using algorithms based on neighbour-joining (Saitou & Nei 1987) and UPGMA (Sneath & Sokal 1973). Bootstrap analysis was used to evaluate the degree of support for individual branches. Population-based analyses employed estimates of allele frequencies as previously described. Gene frequencies were converted into distance matrices using both Nei's (1972) standard and Cavalli-Sforza & Edwards' (1967) chord distance. Cavalli-Sforza chord distance has been found in simulations to outperform other distance measures in phylogeny reconstruction (Takezaki & Nei 1996). Distance matrices were input into PHYLIP for cluster analysis using neighbour-joining and the Fitch & Margoliash (1967) algorithms. In addition, trees were constructed using continuous maximum likelihood (Felsenstein 1981) based directly on estimated gene frequencies. Bootstrap support was calculated for both neighbour-joining trees. The effect of sample size on phylogenetic reconstruction was also examined using repeated subsampling, given that populations varied substantially in sample size. Specifically, a reduced set of individuals ($n = 2–4$) was sampled without replacement from each population, followed by allele frequency estimation and phylogenetic reconstruction via neighbour-joining.

Nonmetric multidimensional scaling (NCSS 1997, NCSS Statistical Software) was conducted on the matrix of Jaccard's distance for individual plants in Wisconsin and the matrix of Cavalli-Sforza chord distance between all pairs of populations in *O. campestris*.

Population aggregation analysis was performed to identify groups of populations with diagnostic bands (Davis & Manos 1991; Davis & Nixon 1992). Populations that were aggregated that did not differ by the fixed occurrence of at least one band. This method identifies diagnosable groups or phylogenetic species (Davis & Nixon 1992) and has been used to identify units of conservation management in beetles (Vogler & DeSalle 1994).

Chromosome counts

Because ploidy level is a significant source of variation within North American *O. campestris* (Table 1), a chromosome count for var. *chartacea* was obtained. One developing inflorescence was collected from Plainfield Lake on April 29, 2000 and fixed in Carnoy's solution in the field. After 3 days, the inflorescence was washed in three changes of 70% ethanol, with an hour or more between changes, and then drained. The inflorescence was then immersed in Snow's acidic carmine solution (Snow 1963) for 4 days to prestain the material. Anthers were dissected from buds in 45% aceto-carmine and transferred to a fresh drop of aceto-carmine. One drop of Hoyer's mounting medium was added before squashing. Anthers giving counts had lengths of 4 mm. Counts were made using a Zeiss phase-contrast microscope. A permanent slide is stored at the University of Wisconsin herbarium.

Morphometric data

Welsh (1991, 1995, 2001) mentions the existence of specimens of '*johannensis*' from Ontario that resemble *chartacea* in pod length. Unfortunately, it proved impossible to obtain sufficient locality or other data for these specimens to include them in the AFLP analysis. To identify these collections and better characterize morphological variation in *O. campestris* var. *chartacea* in Wisconsin and var. *johannensis* from western Ontario, eastern Ontario, Quebec and Maine, we examined specimens from three herbaria (GH, CAN, WIS). Two characters were measured, the length of fruiting pods and the number of flowers per raceme. Length of the fruiting pod has been the most important character used to separate var. *johannensis* and var. *chartacea*; the number of flowers per raceme is a character frequently used to separate varieties of *O. campestris* (Welsh 2001).

Results

AFLP variation

Four primer pairs produced a total of 210 scorable bands (Table 3). All were polymorphic across the entire set of 140 samples, and a unique AFLP profile characterized each individual. Based on 243 samples replicated from final amplification, with 50–66 loci compared per sample, an overall scoring mismatch rate of 0.035 was calculated. The three individuals replicated from field collection had a mismatch rate of 0.039.

Table 4 Genetic diversity measured as the number of rare bands ($f < 0.1$), number of bands unique to a population among those of var. *chartacea*, and among all *Oxytropis campestris* populations; expected heterozygosity (H_E) using *chartacea* loci; H_E using all *O. campestris* loci; nucleotide diversity (π); dissimilarity (D); the probability that two genes share a common ancestor within a population (F). Also shown are estimates of diversity measures using only bands greater than 250 bp in length. Standard errors are given in parentheses

Population	*n*	Rare bands	Private bands (WI)	Private bands (all)	H_E (WI)	H_E (all)	(π) ($\times 10^{-2}$)	*D*	*F*	H_E (WI> 250bp)	H_E (all > 250bp)	(π) ($\times 10^{-2}$) (all > 250bp)	D (all > 250bp)
Oxytropis campestris var. *chartacea*													
Plainfield Lake	25	23	7	6	0.16 (0.015)	0.13 (0.013)	1.3	0.23 (0.008)	0.111 (0.016)	0.15 (0.019)	0.12 (0.017)	1.4	0.27 (0.011)
Second Lake	14	10	1	1	0.15 (0.014)	0.12 (0.012)	1.4	0.23 (0.015)	0.117 (0.019)	0.14 (0.019)	0.12 (0.016)	1.6	0.28 (0.023)
WeymouthLake	25	13	3	1	0.15 (0.014)	0.13 (0.013)	1.5	0.23 (0.005)	0.138 (0.018)	0.15 (0.020)	0.13 (0.017)	1.6	0.27 (0.009)
Huron Lake	4	—	—	2	0.16 (0.014)	0.14 (0.012)	1.7	0.25 (0.046)	0.064 (0.014)	0.15 (0.018)	0.13 (0.016)	1.7	0.26 (0.082)
Portage Co.	4	—	10	3	0.19 (0.015)	0.16 (0.013)	1.9	0.33 (0.00)	0.165 (0.020)	0.19 (0.020)	0.16 (0.017)	2.0	0.38 (0.00)
Bayfield Co.	24	15	20	8	0.15 (0.013)	0.12 (0.012)	1.5	0.26 (0.008)	0.235 (0.023)	0.17 (0.019)	0.14 (0.016)	1.8	0.32 (0.013)
Oxytropis campestris													
var. *dispar*	6	—	—	7	—	0.13 (0.013)	2.0	0.36 (0.015)	—	—	0.11 (0.015)	2.0	0.52 (0.017)
var. *spicata*	4	—	—	3	—	0.11 (0.012)	2.0	0.39 (0.044)	—	—	0.10 (0.014)	2.1	0.47 (0.089)
var. *johannensis* (ONT)	6	—	—	1	—	0.14 (0.014)	1.7	0.30 (0.093)	—	—	0.12 (0.017)	1.8	0.32 (0.080)
var. *johannensis* (ME)	4	—	—	1	—	0.14 (0.013)	1.8	0.28 (0.016)	—	—	0.13 (0.017)	2.1	0.35 (0.000)
var. *johannensis* (NF)	10	—	—	2	—	0.12 (0.013)	1.5	0.27 (0.018)	—	—	0.12 (0.017)	1.8	0.32 (0.020)
var. *jordalii*	2	—	—	1	—	0.10 (0.011)	2.4	—	—	—	0.10 (0.014)	2.6	—
var. *varians*	2	—	—	3	—	0.13 (0.012)	1.5	—	—	—	0.11 (0.015)	1.8	—
Oxytropis borealis	2	—	—	0	—	—	1.9	—	—	—	—	—	—
Oxytropis lambertii	4	—	—	5	—	—	1.8	0.34 (0.041)	—	—	—	2.1	0.44 (0.095)
Oxytropis deflexa	2	—	—	5	—	—	0.5	—	—	—	—	—	—

Table 5 AMOVA for 96 individuals of *Oxytropis campestris* var. *chartacea* from six populations using 210 AFLP markers. The three-level AMOVA includes an additional regional level, corresponding to the three counties where populations are found. The *P*-value is the probability of obtaining an equal or more extreme value by chance alone, estimated from 1023 permutations

Source of variation	df	SSD	Variance component	% of total	*P*-value
Two-level:					
Among populations	5	346.9	3.8	22.5	< 0.001
Within populations	90	1169.7	13.0	77.5	< 0.001
Three-level:					
Among regions	2	228.04	3.69	20.1	0.065*
Among populations/within regions	3	118.84	1.72	9.3	< 0.001
Within populations	90	1169.74	13.00	70.6	< 0.001

*Equals the probability of obtaining an equal value under the null hypothesis; the probability of obtaining a greater value was < 0.001.

Table 6 Global estimates of genetic differentiation among all populations of *Oxytropis campestris* and among populations of variety *chartacea* as Φ_{ST}, F_{ST}; and corresponding estimates of migrants per generation (*Nm*) calculated from F_{ST} estimates using island and stepping-stone models

Populations:	Φ_{ST}	F_{ST}	*Nm* (island)	*Nm* (stepping-stone)
all *O. campestris* (19)	0.30	0.27	0.7	4.0
chartacea (6)	0.23	0.12	1.8	—

Genetic diversity

Estimates of within-population genetic diversity are summarized in Table 4. Expected heterozygosity (H_E) varied from 0.145 to 0.189 in Wisconsin. Across all taxa, comparisons of 95% confidence intervals found no significant differences in H_E between any pairs of populations either within or between taxa. Both nucleotide diversity and dissimilarity measures however, indicated slightly lower diversity in Wisconsin populations compared with those further west. The Bayfield population and three of four populations from Waushara County had significantly lower dissimilarity values than varieties *spicata* and *dispar*. Bootstrapped samples of *chartacea* had significantly more private bands (18.0 ± 0.38 SE) than *johannensis* samples of the same size (10.6 ± 0.47) ($P < 0.012$). The proportion of singleton bands (0.12) among 1000 bootstrapped *chartacea* samples of $n = 20$ was also greater than that found in the 20 *johannensis* samples (0.08), although the relationship was not significant ($P = 0.085$).

A two-level AMOVA (Table 5) of 95 individuals in six Wisconsin populations partitioned 77.5% of the total variation within populations and 22.5% among populations. A three-level AMOVA (Table 5) partitioned 20% among the three regions/counties, 29% of genetic variation among populations, and an additional 9% among the four populations from Waushara County.

A two-level AMOVA on all 19 populations of *Oxytropis campestris* (Table 6) found a similar amount of among-population variation (30%). Allele frequency-based estimates of F_{ST} found only 12% of variation to be held among the six Wisconsin populations while 27% was estimated among all 19 populations of *O. campestris*. Estimates of *Nm* based on global F_{ST} (Table 6) were 0.70 for all *O. campestris* populations and 1.8 for *chartacea* populations under an island model. The estimate of *Nm* among all populations of *O. campestris* using a stepping-stone model was considerably higher at $Nm = 4$. Among Wisconsin populations, the value of the statistic *F* appeared to substantially reflect physical isolation, with the most geographically isolated site (Bayfield) showing the highest value (0.24). Average pairwise estimates of AFLP divergence among selected populations using several measures were summarized in Table 7.

Table 7 Average pairwise AFLP divergence between *Oxytropis* populations sampled in this study, including Φ_{ST}, F_{ST}, *Nm* according to an island model, Cavalli-Sforza chord distance, and Nei's unbiased genetic identity

Populations	Φ_{ST}	F_{ST}	*Nm*	Genetic distance	Nei's ID
4 Waushara Co. populations	0.12	0.05	4.8	0.04	0.98
3 var. *chartacea* regions	0.29	0.16	1.3	0.11	0.96
3 var. *johannensis* regions	0.27	0.10	2.2	0.08	0.95
var. *chartacea* vs. var. *johannensis*	0.33	0.24	0.8	0.17	0.91
6 varieties of *O. campestris*	0.32	0.27	0.7	0.19	0.94
4 species of *Oxytropis*	—	0.42	0.3	0.34	0.86

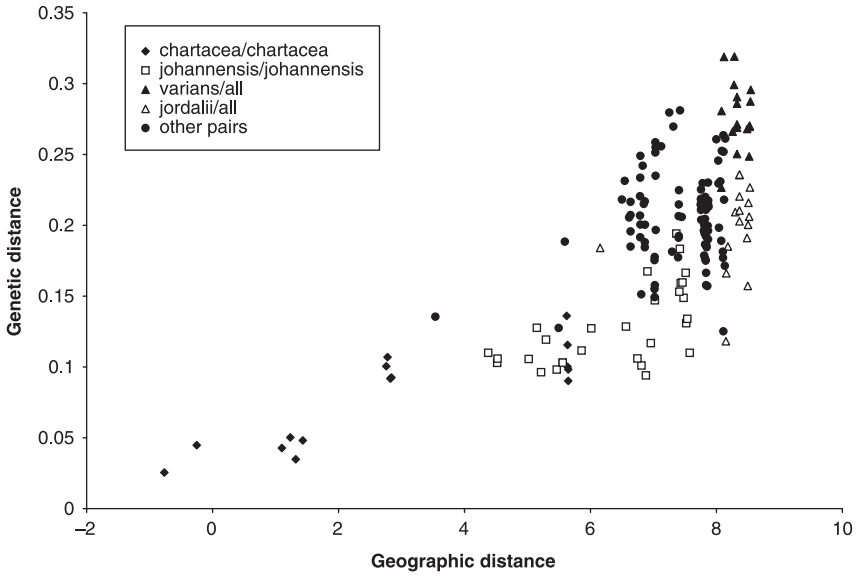

Fig. 3 Cavalli-Sforza chord genetic distance vs. ln geographical distance for *Oxytropis campestris*.

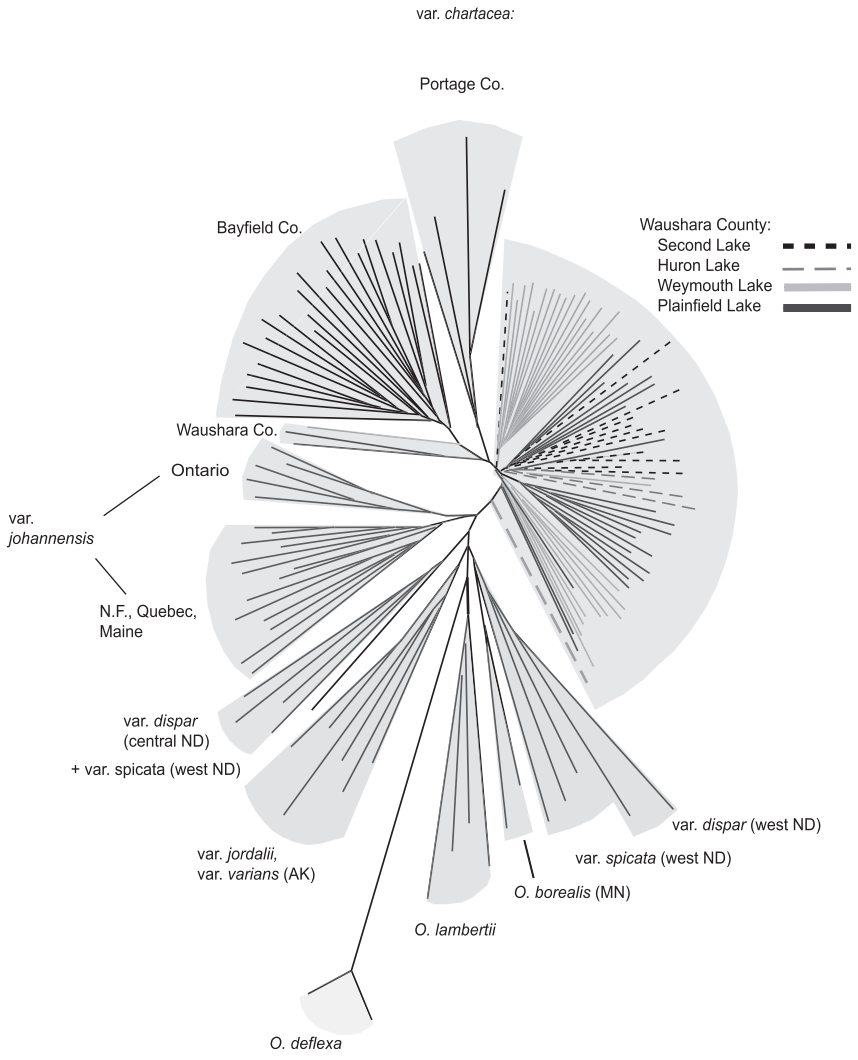

Fig. 4 Neighbour-joining tree based on Jaccard's distance between 140 individuals sampled across 19 populations of *Oxytropis campestris* and one population each of *Oxytropis borealis*, *Oxytropis lambertii*, and *Oxytropis deflexa*.

Spatial autocorrelation

Genetic distance between pairs of *O. campestris* populations increased significantly with geographical distance in all tests with the strongest relationship ($r^2 = 0.63$, $P = 0.0001$) found in the test of Cavalli-Sforza distance and the logarithm of geographical distance between 14 pooled populations (Fig. 3; Table 8). Mantel tests performed on the six Wisconsin populations were significant in three of four analyses. The comparison with the highest r^2 was again that between Cavalli-Sforza distance and log geographical distance ($r^2 = 0.73$; $P < 0.003$).

Phylogenetic relationships

O. campestris var. *chartacea* was monophyletic and sister to *O. campestris* var. *johannensis* in both individual and population-based phylogenetic analyses (Figs 4 and 5). Bootstrap support for the monophyly of both varieties at the population level was 100%, with 86% support for their being sister to each other (Fig. 5). Subsampling of reduced sets of individuals from larger population samples suggested that the structure of the population phylogeny was largely robust to sample size variation and was not greatly affected by the small sample sizes in particular populations. Trees of individuals (Fig. 4) bore long-terminal branches and very short branch lengths at deeper nodes joining clusters of individuals, illustrating the large degree to which AFLP variation is contained within populations. The individual and population-based analyses did not consistently identify which taxon is most closely related to *johannensis*/*chartacea*. However, overall there was a strong tendency for relationships to fall out in a pattern consistent with geographical proximity. Samples of *O. borealis* (MN) fell among

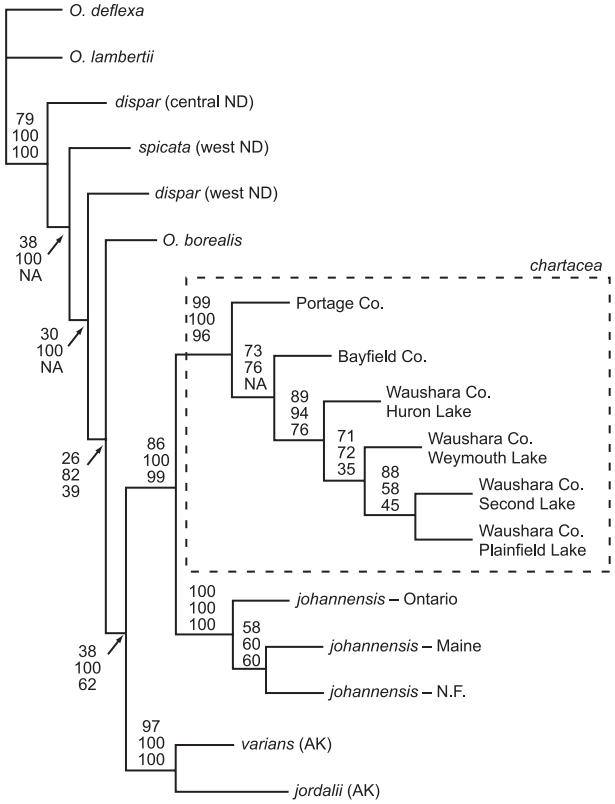

Fig. 5 Neighbour-joining phenogram based on Cavalli-Sforza chord distances between 14 populations of *Oxytropis campestris* and three outgroups. Bootstrap percentages based on 400 resamplings are shown for each clade (top number), followed by the percentage of cases in which the clade is retained under 100 random subsamplings of four individuals (middle number) or two individuals (bottom number) from within population samples of larger size. NA signifies nodes at which the consensus structure is altered under resampling.

Table 8 Results of Mantel tests of geographic vs. genetic distance

Populations	Genetic distance metric*	Geographic distance	r^2	*P*-value
All *O. campestris*	Nei	km	0.55	< 0.001
populations (14)-	Nei	ln km	0.56	< 0.001
populations pooled	CS	km	0.51	< 0.001
	CS	ln km	0.63	< 0.001
All *O. campestris*	Nei	km	0.43	< 0.0001
populations (19)-	Nei	ln km	0.51	< 0.0001
populations separate	CS	km	0.40	< 0.0001
	CS	ln km	0.54	< 0.0001
Wisconsin populations (6)	Nei	km	0.35	0.19
	Nei	ln km	0.66	0.04
	CS	km	0.44	0.02
	CS	ln km	0.73	0.00

*Nei = Nei's distance (1972), CS = Cavalli-Sforza and Edwards' (1967) chord distance.

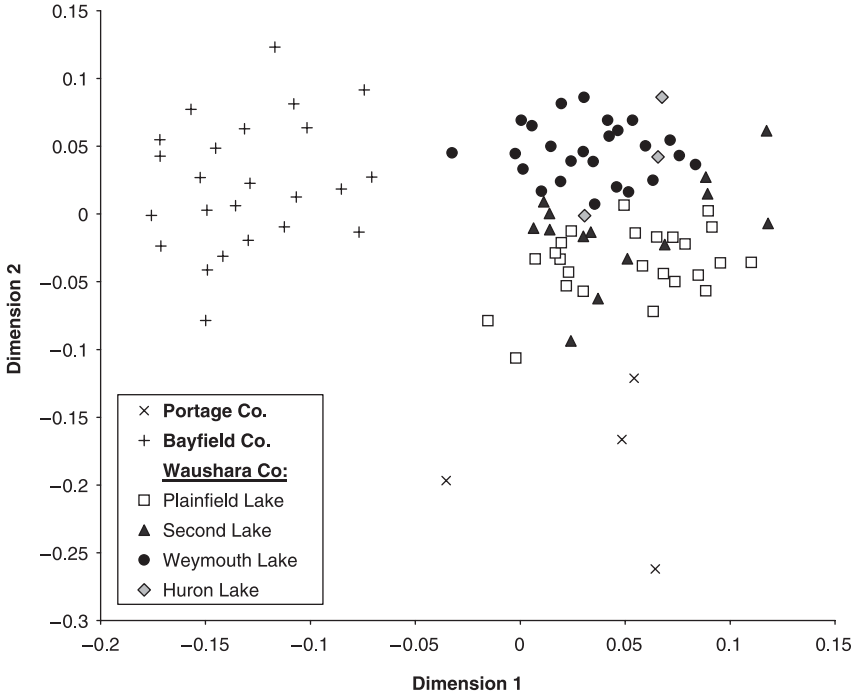

Fig. 6 Nonmetric multidimensional scaling analysis of Jaccard's distance between 95 individuals of *Oxytropis campestris* var. *chartacea* from the six populations in Wisconsin. The first two axes explain 52.5% of total variation.

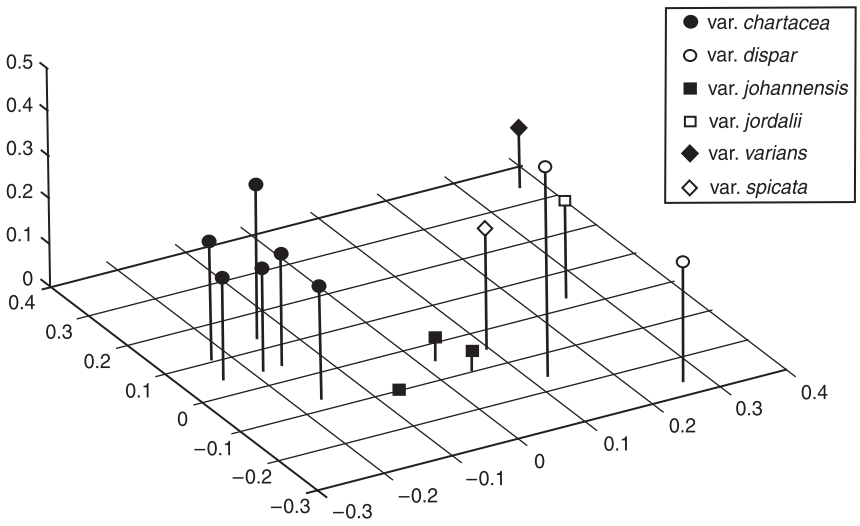

Fig. 7 Nonmetric multidimensional scaling analysis of Cavalli-Sforza chord distance between 14 populations of *O. campestris*. The first three axes together explain 79.6% of the variation.

O. campestris taxa in all analyses, clustering with samples of *O. campestris* vars. *spicata* and *dispar* (ND).

Population aggregation analysis found no fixed differences among a group of populations including *O. campestris* var. *chartacea*, var. *johannensis*, var. *spicata*, var. *dispar*, and *O. borealis*, despite small sample sizes biasing this analysis toward the recognition of more fixed differences than might actually exist. *O. campestris* var. *jordalii*, *O. campestris* var. *varians*, *O. lambertii*, and *O. deflexa* populations did not aggregate with any other populations.

The nonmetric multidimensional scaling analysis of Jaccard's distance among individuals in Wisconsin revealed genetic differentiation of plants from each of the three geographical regions there (Fig. 6). The first two axes explain 52.5% of the genetic variation, and separate the 96 individuals into two large clusters representing Bayfield and Waushara populations. The four Portage county samples are separate from these clusters but do not themselves cluster tightly.

The first two axes of the nonmetric multidimensional scaling analysis of Cavalli-Sforza pairwise chord distances across populations of *O. campestris* explained 65.5% of the genetic variance. Populations of var. *chartacea*, var. *johannensis*, and the two varieties from North Dakota form separate clusters, with the Wisconsin and *johannensis* clusters being adjacent to each other (Fig. 7).

Chromosome count

A diploid count of $2n = 32$, tetraploid in the *O. campestris* complex, was obtained for *O. campestris* var. *chartacea*. Only vars. *dispar*, *spicata*, *davisii*, *jordalii*, and *varians* share this chromosome number (Table 1). Together, these tetraploids form a chain running from *varians* in the northwest to *chartacea* in Wisconsin (Fig. 2).

Morphometric data

Examination of herbarium specimens identified as *O. campestris* var. *johannensis* and *chartacea* found significant differences in pod length in all pairwise comparisons of the four regions examined, except eastern Ontario vs. Quebec/Maine ($P = 0.56$). The mean number of flowers per raceme differed significantly in all regional comparisons except that between eastern Ontario and Quebec/Maine ($P = 0.19$) and between *chartacea* and western Ontario ($P = 0.10$)

Plants near the south shore of Hudson Bay in western Ontario (Fig. 2), specifically from areas along the Severn, Fawn, Poplar, and Black Duck Rivers, as well as a sample from the shores of Hudson Bay itself near Winisk differ substantially from other samples of variety *johannensis* in eastern Ontario and Quebec/Maine. Plants from the Hudson Bay region more closely resemble variety *chartacea* from Wisconsin in pod length and number of flowers per raceme than they do variety *johannensis* from other regions (Fig. 8). Interestingly, all but one plant from western Ontario had a predominance of fasciculate leaflets (more than two arising from a single point on the rachis), a character otherwise missing in var. *chartacea* and var. *johannensis*. Elsewhere in the *O. campestris* complex this character-state is found only in *O. campestris* var. *davisii* and *O. splendens* from the Rockies.

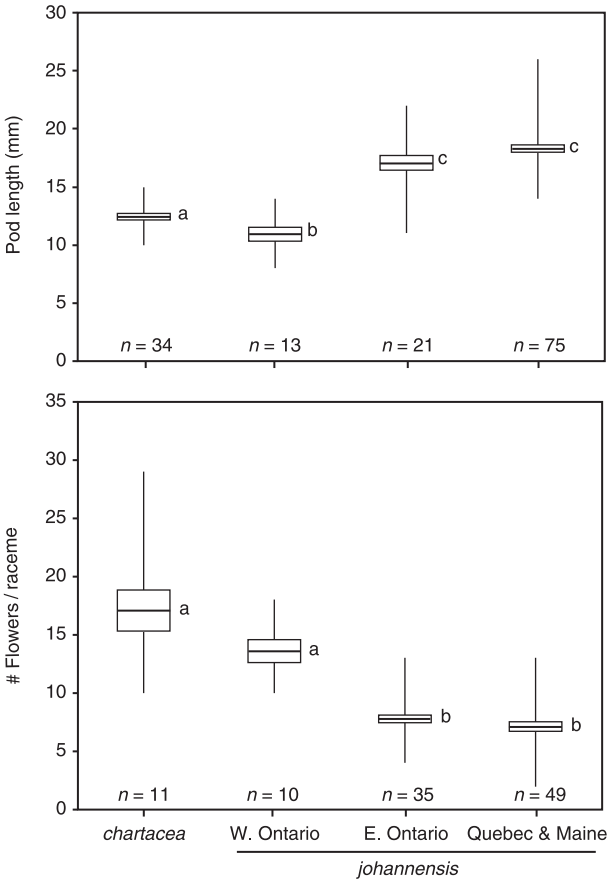

Fig. 8 Pod length and numbers of flowers/raceme in herbarium specimens of *Oxytropis campestris* var. *chartacea* and var. *johannensis* from western Ontario, eastern Ontario, and Gaspé peninsula, Quebec/St. John River, Maine (see Fig. 2). Box plots show mean ± SE, with lines extending to the extreme values observed for each region. Means that are significantly different from each other do not share any letters; sample size is indicated below each plot.

Discussion

Genetic diversity in Fassett's locoweed

A comparison of AFLP diversity in Fassett's locoweed vs. other plant species suggests that it retains relatively high levels of genetic variation among individuals, but relatively little variation among populations. Expected heterozygosity within the six populations of Fassett's locoweed ($H_E = 0.145–0.189$) falls at the high end of estimates in plant studies using AFLPs, from $H_E = 0.0024$ in the apomictic *Limonium dufourii* (Palacios *et al*. 1999) to $H_E = 0.23$ in a population of *Eryngium alpinum*, a long-lived, insect-pollinated perennial (Gaudeul *et al*. 2000). Nucleotide diversities also indicate a fairly high level of genetic variation within populations of Fassett's locoweed ($P = 0.0132–0.0191$), higher than that estimated for *Dioscorea tokoro* ($P = 0.0023$; Innan *et al*. 1999; Borowsky 2001), but within the range calculated from 10 different AFLP primer pairs for 38 ecotypes of *Arabidopsis thaliana* ($P = 0.0068–0.0199$) (Miyashita *et al*. 1999). AMOVA results for RAPD studies on outbreeding vs. selfing plants have been summarized by Bussell (1999). Plants classified as outbreeders had Φ_{ST} values ranging from 0.009 to 0.413, while values for inbreeders ranged from 0.45 to 0.67. Fassett's locoweed ($\Phi_{ST} = 0.23–0.29$) thus falls in the middle of the range for outbreeding plants.

Breeding system is the most powerful explanatory variable for genetic diversity within and among populations of plant species (Hamrick & Godt 1989), and our results are consistent with what is known about the breeding system of Fassett's locoweed: primarily outcrossing, if not obligately so. Pilot studies conducted as part of the current investigation indicate that self-fertilization and apomixis are absent or rare (< 2% of flowers within bagged inflorescences set fruit), confirming results from a previous study

on *O. campestris* var. *spicata* in Montana (Bauer 1983). Other characteristics of Fassett's locoweed consistent with high gene flow potential and high within-population variation include a polycarpic, perennial habit, long seed dormancy, seasonal, synchronous flowering and pollination by large bees (Loveless & Hamrick 1984).

Gene flow estimates corresponding to the level of population differentiation found in Fassett's locoweed (Table 6) are high, well in excess of that generally considered to counteract drift. While consistent with the breeding system of this plant, these results are surprising given the current isolation of each population and its gravity-dispersed seeds. The observed pattern most likely reflects past gene flow, consistent with current populations of Fassett's locoweed being relicts of a more widespread distribution during the Pleistocene. The strong correlation between genetic and geographical distances between populations (Table 8) and the substantial number of private and singleton bands (Table 4) indicate that long-distance dispersal and accompanying bottlenecks have been rare. These results are consistent with gradual migration and a formerly more continuous distribution for Fassett's locoweed, and inconsistent with its origin via a series of independent, long-distance dispersal events.

Relationship of Fassett's locoweed to other taxa

Patterns of AFLP variation among individuals and populations strongly support the close relationship between *O. campestris* var. *chartacea* and var. *johannensis* proposed by Barneby (1952) on morphological and biogeographical grounds. Phylogenetic and cluster analyses indicate, however, that each of these varieties forms a distinct lineage (Figs 4, 5 and 7).

These results support the continued recognition of Fassett's locoweed as a separate taxon. Arguing against this is an absence of nonoverlapping morphological characters separating *chartacea* from *johannensis*. (Fig. 8). We found high support for the monophyly of each taxon based on AFLPs, but an absence of fixed differences. However, this pattern of genetic similarity extends beyond these two varieties to all taxa of *O. campestris* sampled, even *O. borealis* and to a lesser degree *O. lambertii*. Average Nei's genetic identity among all varieties of *O. campestris* sampled here was high (0.94), higher than the average calculated only between populations of var. *chartacea* and *johannensis* (0.91). Clearly, both vars. *chartacea* and *johannensis* are part of a larger complex currently undergoing differentiation, at least in part, via isolation by distance (Figs 3–5, Table 8). Our single chromosome count for var. *chartacea* indicates that it is tetraploid ($2n = 32$), in contrast with var. *johannensis*, which is consistently hexaploid ($2n = 48$) at the five localities sampled (Ledingham 1960; Gervais & Blondeau 1999). Molecular, phylogenetic, and cytological data thus suggest that the continued recognition of these entities at the subspecific level is indeed justified.

This finding contrasts with two recent studies of subspecific taxa in the *O. campestris* complex in the Alps (Schönswetter *et al*. 2004) and unglaciated Alaska (Jorgensen *et al*. 2003), which failed to find a genetic basis for recognizing the taxa in question. In the Alps, endemic *O. campestris* ssp. *tiroliensis* had been separated from the widespread ssp. *campestris* on the basis of flower colour and minor differences in the sizes of the standard and calyx. Analyses of AFLPs and morphology, however, showed no significant separation between the two entities except flower colour. In Alaska, individuals of seven subspecific taxa assigned to *O. campestris* and *O. arctica* with varying ploidy levels were intermingled in trees based on ITS (internal transcribed spacer) and RAPD (random amplification of polymorphic DNA) data. For these plants, geography, which splits the populations into two groups, appeared to be a better predictor of genetic relatedness than taxonomic designation or shared morphological traits. Jorgensen *et al*. (2003) concluded that neither morphology nor molecular characters can be used as the basis for a reliable taxonomy of these polyploid taxa because of their multiple origins, widespread hybridization, and lack of time since their origin in the Pleistocene for the fixation of traits.

Reconstructing the history of Fassett's locoweed

A likely scenario for the rise of *chartacea* and *johannensis* includes the initial migration eastward of a tetraploid ancestor during the Pleistocene along a periglacial corridor from sparsely covered, calcareous arctic tundra to the north or Alpine tundra in the Rockies. This could have been followed by isolation of *chartacea* in or near the Wisconsin glacial refugium, followed by the polyploid origin of *johannensis* during the same or subsequent glacial cycle, in the same area or a different refugium/periglacial corridor to the east. The fact that *johannensis* is sister to *chartacea*, rather than embedded within it, argues for a nearly simultaneous origin of both varieties in different peripheral isolates (see below), or perhaps for extinction of ancestral *chartacea*-like forms related to *johannensis* that, in turn, left modern *chartacea* monophyletic. In the unglaciated Driftless Area of Wisconsin, treeless communities existed from 23 000 to 15 000 years ago, and persisted along the glaciers' periphery until 11 400 years ago (Pewe 1983; Clayton *et al*. 2001). The open, calcareous habitat near the constantly shifting glacial margin would have provided large expanses of suitable habitat for Fassett's locoweed, given its extremely short vegetative stature and expected restriction to sparsely covered microsites (Givnish 1982, 1995) and the general restriction of *O. campestris* to calcareous tundra or cool shoreline communities (Welsh 2001). The abundance of waterways and their partly open shores in newly deglaciated

terrain would have facilitated the initial spread of vars. *chartacea* and *johannensis* and/or their common ancestor. Subsequently, both forms would have been extirpated where open, calcareous habitats disappeared.

The disjunctions in var. *johannensis*' current range (Fig. 2) correspond, at least in part, to disjunctions in calcareous bedrock in northeastern North America. Soon after glaciation, cation-rich glacial drift would have been briefly widespread even over granitic bedrock, facilitating the spread of *johannensis* and ecologically similar taxa. Richard (1974, 1989) has shown that the calciphilic *Shepherdia canadensis* is now widely disjunct and restricted to calcareous substrates in Quebec-Labrador, but based on its pollen record, was ubiquitous soon after glaciation. Pollen records indicate that *Astragalus alpinus* (with a distribution remarkably like that of *O. campestris*) and an unidentified *Oxytropis* were common far east of their current distribution from 20 500 to 14 700 years ago (Birks 1976).

Saxifraga oppositifolia, a widespread, calciphilic, arctic-alpine cushion herb, show a less marked, perhaps more recent stage of genetic divergence in response to glacial retreat (Gabrielsen *et al*. 1997). Its patchy distribution in Norway exhibits a north–south cline in RAPD variation but little genetic structure, providing evidence of abundant gene flow between now-disjunct populations. Fossils indicate that this species was widespread periglacially during the last glacial period, following the ice sheets northward as they retreated, but then excluded outside alpine tundra by competition. Although var. *chartacea* can reach sexual maturity in a few years, its long-lived seed bank and overlapping generations should increase generation time and slow its tempo of genetic differentiation (see Templeton & Levin 1979).

Our sampling of the *O. campestris* complex failed to identify the sister group to the *chartacea-johannensis* clade. Other tetraploid varieties include *varians*, *jordalii*, *davisii*, *spicata*, and *dispar*, forming a chain from *varians* in Alaska in the northwest to *chartacea* in the southeast (Fig. 2). Given that our data support a strong correlation between geographical and genetic distance among the taxa surveyed (Table 8), this geographical pattern may represent differentiation of populations isolated in adjacent refugia during glacial maxima and/or isolation by distance as part of a general movement from Beringia eastward along periglacial corridors during those maxima. The low support for nodes deep in the individual and population-based trees, and the conflict in their topologies, might reflect small sample sizes and incomplete sampling of the complex. But these results might also reflect the simultaneous divergence of several peripheral isolates from a wide-ranging, high-latitude ancestor—an expected consequence of the expansion of the Laurentian glaciers and isolation of populations in different refugia along their margins. The lack of resolution deep in the tree may also reflect widespread hybridisation. Relationships within polyploid complexes can be complicated, involving multiple formations of particular combinations, and repeated cycles of range expansion and contraction causing secondary contact and gene flow between previously separated populations, both within and between ploidy levels (Stebbins 1984; Soltis & Soltis 2000; Abbott & Brochmann 2003). Phylogenetic analyses that assume a bifurcating tree may be unable to infer relationships accurately if any of these processes are common.

Morphometric analyses confirmed reports in the literature of specimens labelled '*johannensis*' from western Ontario that resemble *chartacea* in pod length. Our data suggest that the western Ontario populations resemble *chartacea* more than they do *johannensis* in pod length and flower number. This raises the possibility that *chartacea* may not be restricted to Wisconsin. However, arguing against this is the fact that these plants have fasciculate leaflets, missing in both *chartacea* and *johannensis* but seen in some western varieties. Unfortunately, specimens from this region were not a part of our AFLP analysis and a better characterization of their place in the *O. campestris* complex must thus await chromosome counts and their inclusion in a more extensive genetic analysis. Our documentation of the actual location and the morphological distinctiveness of the material from western Ontario make such a study now feasible.

Conclusions

Although the dominant AFLP markers provide no measure of inbreeding, the observed levels of genetic variation give no reason to suspect that genetic factors are an overriding management concern. Given the unusual habitat requirements of Fassett's locoweed, we believe that ecological changes rather than genetic loss pose a greater risk in the near future. The existence of high levels of genetic diversity (possibly maintained by seed banks) despite very small numbers of plants above-ground suggests the potential for extirpation because of ecological causes. If conditions that allow germination or seedling establishment, i.e. significant lake-level fluctuations, accompanied by slow rates of revegetation by taller competitors are absent for too long, seed viability will be lost and, with it, the local populations of Fassett's locoweed. Preserving the natural hydrological regime of the lakes supporting this endangered plant, and the low substrate fertility and undeveloped nature of the lakeshores it occupies are the most important precautions that should be taken to ensure its continued survival.

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